(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 351 248 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication of patent specification: **22.11.95**

(51) Int. Cl.[6]: **G01N 33/53**, G01N 33/543, G01N 33/569, G01N 33/545, G01N 33/552

(21) Application number: **89307202.5**

(22) Date of filing: **14.07.89**

The file contains technical information submitted after the application was filed and not included in this specification

(54) **Detection of a feline pathogen-specific antibody and a feline pathogen antigen in an immunoassay.**

(30) Priority: **14.07.88 US 219100**

(43) Date of publication of application:
17.01.90 Bulletin 90/03

(45) Publication of the grant of the patent:
22.11.95 Bulletin 95/47

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(56) References cited:
EP-A- 0 171 150
EP-A- 0 173 295
EP-A- 0 200 381
EP-A- 0 286 264
GB-A- 2 051 357
US-E- 32 696

AMERICAN JOURNAL OF VETERINARY RESEARCH, vol. 49, no. 8, August 1988, Chicago, IL (US); J.K. YAMAMOTO et al., pp. 1246-1258

(73) Proprietor: **IDEXX LABORATORIES, INC.**
100 Fore Street
Portland, ME 04101 (US)

(72) Inventor: **O'Connor, Thomas P.**
27 Baywood Lane
Yarmouth, ME 04096 (US)
Inventor: **Tonelli, Quentin J.**
37 Wellington Road
Portland, ME 04103 (US)

(74) Representative: **Hitchcock, Esmond Antony**
Lloyd Wise, Tregear & Co.
Norman House
105-109 Strand
London WC2R 0AE (GB)

EP 0 351 248 B1

Description

This invention concerns immunoassay methods for the detection or measurement of substances in liquid samples, e.g., biological fluids such as whole blood, serum, plasma, and urine.

A wide variety of substances are commonly detected or measured by immunoassay methods, for example, hormones, antibodies, toxins, drugs, and antigens such as viral particles. Usually, although not always, either the substance being detected or a substance used in its detection is an antibody, hence the term "immunoassay". The antibody is a member of a specific binding pair, the other member of the pair being referred to as an antigen, or analyte. Other specific binding pairs, besides antibodies-antigen pairs, which are measured and used in similar assays, include pairs of molecules which have specific binding affinity for each other, e.g., hormones and hormone receptors, and biotin and avidin.

Immunoassays are commonly carried out, at least in part, on solid supports, e.g., glass fiber membranes. The two most common formats for immunoassays employing solid supports are competitive and sandwich formats. Typical competitive formats are described e.g., in Littman et al., U.S. Patent 4,540,659, and a typical sandwich assay by David et al., U.S. Patent 4,376,110.

Immunoassays may be performed to detect more than one substance in a sample at the same time. EP 200381 refers to a solid phase system with distinct groups of microspheres entrapped in a porous matrix. Different groups may be bound with different receptors to facilitate a multiple assay to detect two or more analytes. The system disclosed in EP 171150A includes a solid support having a plurality of receptors bound to it, which may be used to assay simultaneously for more than one analyte. EP 0 286 264 discloses an assay for HBsAg and HIV anti bodies comprising a solid support on which are bound a peptide having an epitope capable of binding to an HIV antibody and an antibody to HBsAg. The materials from the sample bind to the solid support and are detected by means of a second labelling reagent. GB 2051357 provides a method for detecting at least two indicators of exposure to Hepatitis virus, by immunoassay.

The simultaneous detection of antigen and antibody in a serum employing two assays on a single solid support is described in EP 173295A. However, false positive results may occur as assays for sample antibodies are usually conducted at a higher sample dilution than in a standard antigen sandwich assay. The dilution is required to avoid the binding of irrelevant sample antibodies to the solid support.

Examples of the use of a sandwich test are an immunofluorescent assay (IFA) and an enzyme-linked immunosorbent assay (ELISA). Both these assays are used for detection of a common disease of cats, feline leukemia virus (FeLV).

FeLVs are an endogenously replicating C-type ocornavirus; the viral genome is incorporated into the host chromosome as a provirus, and the genome is translated to produce intact virions. The virus is spread horizontally from infected to susceptible cats and causes a number of disease syndromes ranging from myeloid and lymphorecticular neoplasms to an acquired immunodeficiency syndrome.

Another virus which infects cats is a retrovirus recently isolated from a group of cats suffering from immunodeficiency-like syndrome; the virus is termed feline-T-lymphotrophic lentivirus (FTLV or FIV). It belongs to the same group as the human immunodeficiency virus (HIV), the causitive agent of human AIDS. FIV is described by Pederson et al., 235 Science 790, 1987. Antibody to FIV has been detected by use of an IFA.

The present invention provides a method for detecting a feline pathogen antigen and a feline pathogen-specific antibody in a biological sample, comprising:

(a) providing a feline pathogen antigen which selectively forms a first immune complex with a sample antibody, the antigen being directly bound to a solid support at a first location;

(b) providing a feline pathogen-specific antibody which selectively forms a second immune complex with a sample antigen, the antibody being directly bound to the solid support at a second location;

(c) contacting the first location on the support with at least a portion of a biological sample under conditions whereby the first immune complex can form and contacting the second location on the support with at least a portion of such sample of equal concentration under conditions whereby the second immune complex can form;

(d) washing unbound material from the first and second locations;

(e) detecting whether the first immune complex is formed by adding a labelled antigen which selectively binds thereto; and

(f) detecting whether the second immune complex is formed by adding a labelled antibody which selectively binds thereto.

Preferably the antigen is FeLV and the antibody is anti-FIV or alternatively, the antigen is FIV and the antibody is anti-FeLV.

Also in accordance with the invention, a solid support is provided on which an immunoassay of the invention may be executed. The feline

pathogen antigen is directly bound to the support at a first location and the feline-specific antibody is directly bound to the support at a second location.

Preferably the solid support comprises one of a microtiter well, a glass or plastic bead, a filter matrix, a polystyrene latex bead, or other microparticles. The antigen may be FIV, a polypeptide comprising an epitope of FIV, or derived from FIV, or substantially the same as a polypeptide derived from FIV, and the antibody may be anti-FeLV. By substantially the same is meant that the polypeptide has an amino acid sequence identical to, or with less than 10% substitutions, compared to the naturally occurring sequence.

Alternatively, the antigen is FeLV, a polypeptide comprising an epitope of FeLV, or derived from FeLV, and the antibody is anti-FIV.

According to another aspect of the invention a kit is provided comprising:

(a) a solid support comprising a feline pathogen antigen capable of selectively forming a first immune complex with a sample antibody, which antigen is directly bound to a solid support at a first location; and a feline pathogen-specific antibody capable of selectively forming a second immune complex with a sample antigen, which antibody is directly bound to the solid support at a second location, wherein the antigen and the antibody are each capable of forming separately detectable immune complexes with biological samples of equal concentration;

(b) means for detecting said first complex comprising a labelled antigen which selectively binds thereto; and

(c) means for detecting said second immune complex comprising a labelled antibody which selectively binds thereto.

Preferably the antigen is FIV, a polypeptide comprising an epitope of FIV, or derived from FIV, and the antibody is anti-FeLV, or alternatively, the antigen is FeLV, a polypeptide comprising an epitope of FeLV, or derived from FeLV, and the antibody is anti-FIV.

In a preferred embodiment, the solid support has a region including one or both of a positive and a negative control spot (as explained below).

In an embodiment of the invention a method for detecting the presence of antibody to FIV in a biological sample includes the steps of providing purified FIV, or a purified antigen from FIV, or a purified polypeptide substantially the same as a polypeptide isolated from FIV, wherein the purified FIV is substantially free from FIV host cell proteins and is composed of at least 5% p26 (the major nucleocapsid protein, as measured by densitometric scans of coomassie blue G-250 stained SDS-PAGE) as total protein; binding the purified FIV, or the antigen or the polypeptide to a solid support; contacting the sample with the solid support under conditions under which the antibody can bind the purified FIV or the antigen or the polypeptide, to form a first immune complex; contacting the first immune complex with an enzyme-labelled antibody under conditions under which the detectable antibody forms a second immune complex with the first antibody; and detecting the second immune complex.

The detectable antibody may include an enzyme, e.g., horseradish peroxidase or alkaline phosphatase; and the detectable antibody may be anti-feline antibody.

Preferably, the invention features purified FIV substantially free from FIV-host cell proteins, composed of at least 5% p26 as total protein.

The invention provides a test for detection of FIV in biological samples which has greater specificity and sensitivity than prior tests. By using a labelled antigen able to react with anti-FIV it is possible to form a large immune complex having many labelled molecules; this increases the test sensitivity. The ELISA FIV test of the invention, which, preferably uses a greatly purified FIV antigen, and increases sensitivity and specificity over existing methods of testing for FIV.

Similarly, any pair of antigen and antibody may be simultaneously and accurately detected in a single blood sample by use of a single testing device. This has not previously been possible, using conventional techniques, since for example, an antigen in whole blood requires use of undiluted blood, whilst assay for an antibody requires use of diluted blood. This invention overcomes this problem by using a labelled antigen, rather than a labelled antibody, to detect antibody in the sample. Thus, for example, whole blood of humans may be simultaneously screened for Hepatitis B antigen and HIV antibody and any blood which is positive for either of these disease-causing agents discarded from, for example, a blood bank. This method can be used to rapidly screen large numbers of biological samples for any desired mixture of antigens and antibodies without loss of sensitivity compared to alternative procedures. A single test thus takes the place of a plurality of prior tests.

A simultaneous assay of this type may therefore provide a means for rapidly screening blood or other biological fluids for infective agents, such as FeLV, FIV, Hepatitis antigen, and HIV. Simultaneous screening for mixtures of antigens and antibodies allows a single test to be performed to determine whether or not the biological sample is useful, e.g., whether human blood can be used in a transfusion. The assay also allows determination of the cause of infection by one or more viruses producing similar clinical symptoms, such as FeLV and FIV. It is also possible to perform a simulta-

neous assay for antigens and antibodies associated with the same viral infection, e.g., HIV antigen and anti-HIV antibody. (For this assay it is necessary to provide a monoclonal antibody which recognizes the epitope on the HIV antigen which is chemically bound to the enzyme label, for example, a monoclonal antibody directed against p24 (commercially available) can be used.)

The simultaneous FeLV and FIV test of the invention is useful in determining whether a cat with "Sick Cat Syndrome" is infected with a chronic disease (FIV or FeLV) and thus should be isolated from other cats or perhaps destroyed; or is not so infected and can thus be treated with antibiotics. About 12-15% of such cats are infected with FIV, 12-15% with FeLV, and the remainder are not infected with these viruses. Similarly, the test can be used to test a kitten to determine whether to administer an FeLV vaccine to it, and whether to allow the kitten to come in contact with other cats.

Other features and advantages of the invention will be apparent from the following description of preferred embodiments thereof, and from the claims.

The Figure is an exploded isometric view of an assay device suitable for use in the invention.

Simultaneous Assay for FeLV Antigen and FIV Antibody

The following is an example of simultaneous detection of an antigen and an antibody in a single test. In this example, FeLV antigen and antibody to FIV in feline serum, plasma, or whole blood are measured simultaneously in a single test. In this example, the assay uses a solid phase format for immunoassay of FeLV antigen and FIV antibody; other formats are also suitable, for example, a membrane based format; a microtiter well-based format; the dry format described EP-A-0 327 395; and the format described in EP-A-0 263 978.

Referring now to the Figure, the FIV/FeLV assay is performed using anti-FeLV monoclonal antibody coated latex particles spotted onto glass fiber filter 18 at position 19. Similarly, on the same filter 18, latex particles coated with FIV antigen are spotted for use in the FIV antibody assay at position 20. The incorporation of both positive and negative control reagents at positions 22 and 21, respectively, on filter membrane 18 allows two complete test procedures to be carried out simultaneously, yielded an unambiguous result.

Still referring to the Figure, glass fiber filter 18 is also provided with an orientation spot 24, and is incorporated into cylindrical disposable assay device 10 supported on porous polyethylene disc 16 and cellulose acetate absorbant 14, as described in McMahon et al. U.S. Patent Application Serial No. 145,522, filed January 19, 1988, entitled IMMUNOASSAYS, assigned to the same assignee as the present application and hereby incorporated by reference. The combined wicking properties of these materials serve to control the flow of sample and reagents through the membrane. This system minimizes the kinetic problems associated with solid phase immunoassays in that it eliminates the "unstirred" layer of solution at the surface of the solid phase, in which diffusion limits transport of reagents to reactive particle-bound components. The design of the particulate solid phase and its function within the assay device enhance the binding of reagents compared to other solid phases. This configuration may be used for serum, plasma, or whole blood.

In addition to filter 18, cylinder 12 includes accessory prefilter 26, which serves to remove insoluble material from the sample during application. Device 10 is assembled by placing the component parts described above into cylinder 12 fixed to an upper conical-shaped cup 30. A plunger 32 having a handle 36 and a sponge portion 34 is used to seat each of the component parts.

Generally, the conjugate mixture used to detect any bound antigen or antibody on filter 18 contains horseradish peroxidase (HRPO) conjugated monoclonal antibody to p27 (a major core protein of FeLV) and HRPO conjugated FIV antigen. The conjugate and test sample are mixed, and conjugated monoclonal antibody will bind p27 antigen (if present), while conjugated FIV antigen will bind anti-FIV antibody (if present). Upon application of this mixture to assay device 10, the membrane-bound anti-p27 antibody will capture the p27 conjugated antibody complex, and the membrane-bound FIV antigen will capture the anti-FIV conjugated antigen complex. Following a wash step and addition of substrate and chromogen, color development in the FeLV sample spot indicates the presence of FeLV antigen, and color development in the FIV sample spot indicates the presence of FIV antibody.

A feature of this assay is the incorporation of positive and negative controls. Positive control particles are coated with antibody to HRPO. A small amount of conjugate applied to the membrane during the course of the assay is captured by the positive control spot. Subsequent color development by the positive control indicates that the conjugate is active; this is one criterion for assay validity. The negative control particles are coated with non-specific mouse antibody and purified uninfected host cell antigen. The amount of mouse antibody and host cell antigen coated on these particles is sufficient to mimic "nonspecific" reactions that may occur with either sample particle. The negative control spot is exposed to all assay

reagents in a fashion identical to the sample spots. The negative control spot should remain clear, or less colored than the sample spots for a positive result to be valid. Substantial coloration in the negative control spot indicates a nonspecificity problem and may invalidate the test. User interpretation of assay results is as follows: (1) positive control only develops color, negative result; (2) positive control and FeLV and/or FIV sample spot(s) develop color, positive result for respective sample spot; (3) negative control develops color more intense than sample spot, negative result; (4) no color development in any spot, invalid test; (5) negative control develops color less intense than sample spot(s), positive result for respective sample spot.

The individual components of the assay will now be discussed, and a detailed example of the assay provided.

FIV Virus Antigen

The FIV antigen employed is FIV virus (particles), or is derived from FIV virus particles, as follows. (Synthetic antigens are also suitable in the assay, as are substantially purified polypetides derived from FIV particles.) Master seed virus producing cultures were obtained in the form of a continuous feline cell line infected with FIV Isolate No. 2427 (Petaluma Strain) from Dr. Niels Pederson (University of California, Davis, Calafornia). Other virus cultures can be obtained as described by Pederson, supra, or by Harbour et al., 122, The Veterinary Record 84, 1988. Seed stocks of virus producing cell cultures were obtained by freeze-downs of FIV-infected master seed cells cultures following at least 19 post infection passages in culture. Additional seed stocks of virus producing cultures are obtained by either infection of the continuous feline cell line with FIV master seed virus or by single cell microwell cloning of high level FIV producers from the original FIV infected master seed cell culture. For propogation, master seed virus infected feline cell cultures are inoculated into tissue cell culture flasks. Following growth to a confluent monolayer of cells, tissue culture fluid is harvested at intervals of 2-5 days.

Working seeds virus is produced by propogation by the master seed cell line permanently infected with FIV. An inoculum is added to tissue culture flasks, incubated, and the spent tissue culture fluid harvested. Typically the flasks are incubated at 36°C-38°C for a maximum of 7 days before fluid and cell harvest. The harvested fluid, including cell material, is centrifuged in a high speed centrifuge (Sorval RC-5B or Beckman J2-21) leading to separation of supernatant and cell pellet material. The cell pellet is discarded, and the supernatant culture fluid used to prepare working virus. The clarified supernatant is made 0.5 M in NaCl and 4%-10% in polyethylene glycol (PEG 8000, Sigma). Following overnight precipitation, virus is pelleted and resuspended in buffer (10 mM Tris, 300 mM NaCl. 1mM EDTA, pH 7.5). The virus is then centrifuged (at 13,000 x g for 15 min.) After centrifugation the pellet is discarded and the clarified supernatant centrifuged in a 50%-90% discontinuous gradient of glycerol. Centrifugation is at 75,000 x g for 3 hrs. and the FIV viral band at the interface collected. The band is suspended in buffer and centrifuged at 75,000 x g for 1 hr; the resulting pellet is resuspended in buffer and stored at -70°C.

Polystyrene Particles

Polystyrene particles (0.2-2 µm from Pandex Laboratories) are suspended in 10 mM potassium phosphate buffer, pH 7.2, and mixed with 50-1000 µg of inactivated FIV particles. FIV is inactivated by provision of 0.25% sodium dodecyl sulfate (SDS) and heating at 56°C for 1 hr. The amount of SDS may be varied proportional to the protein content of the FIV particles. Generally, 2.4 mg of SDS are used per mg of antigen. After incubation at 15°C-30°C for 1-24 hrs. the particles are centrifuged at 13,000 x g for 10 min. The pellet is resuspended in 1% Bovine serum albumin (BSA), 10 mM potassium phosphate buffer,pH 7.2 incubated at 15°C-30°C for 0.5-2 hrs., and centrifuged as above. The pellet is washed in buffer and centrifuged again. The resulting pellet is then resuspended in 2.5% sucrose 10 mM potassium phosphate buffer and 0.05% Tween® 20. 2.5-6 µl of a 1% solution of the resulting particles are spotted onto the assay device.

FIV Antigen Conjugate

Purified FIV virus is inactivated by addition of detergent and heat treatment at 65°C for 90 min. Virions are further disrupted by the addition of surfactant. For example, 2 ml of FIV antigen is added to 4.8 mg SDS, heated at 65°C for 90 min., and allowed to cool for 2 hrs. at 4°C. After centrifugation of 4°C for 10 min. in a microcentrifuge tube, Triton® X-100 is added to a final concentration of 1.5% and the mixture allowed to stand at 15°-30°C for 30 min. 0.9 mg of Bio-beads (Bio-Rad S-100, Bio-Rad Laboratories) are added per ml of solution, and the mixture shaken for 2 hrs. The solution is removed and dialyzed overnight against 25 mM $NaHCO_3$, pH9.2. The clarified antigen is then treated with metaperiodate activated horse radish peroxidase by the method of wilson et al. (Immunofluorescence and Related Staining Tech-

niques, ed. Knapp et al., p. 215, 1978), and the conjugate treated with sodium borohydride and diluted in conjugate diluent (10 mM Tris, pH 7.2-7.6 containing 0.05% Tween® 20, 50% calf serum, 10% mouse serum, 0.1 mg D & L Blue dye, 16 mg/l gentamicin (Sigma) and 0.2g/l Thimerosol (Sigma)).

FeLV Antibody

Monoclonal antibody producing cell lines against FeLV virus p27 antigen were obtained from the School of Veterinary Medicine, University of California, Davis, California. These cell lines are available from the University of California. The cell lines are initially propogated in tissue culture and then injected into pristane primed BALB/C or IRC/FI mice. The acites fluid is harvested and clarified. The antibody is partially purified by either ammonium sultate precipitation or column chromatography using affinity or ion exchange gels. Particles are prepared as described above for FIV. The coating buffer contains the mouse antibody to FeLV. Other suitable monoclonal antibodies to FeLV can be prepared by standard techniques from FeLV viral particles (available from Electro Nucleonics Inc., Silver Spring, Maryland).

FeLV Conjugate

Monoclonal anti-FeLV horse radish peroxidase conjugate is prepared as described above for FIV conjugate.

Control Particles

Negative particles as a control for the FIV test are coated with an extract of an uninfected cell line. Negative particles for the FeLV test are coated with IgG non-reactive to FeLV (purchased from Sigma).

Positive particles for the FIV and FeLV tests are coated with anti-horse radish peroxidase as described above. Positive particles are manufactured with anti-horse radish peroxidase purchased from Atlantic Antibodies or from Jackson Laboratories (Pennsylvania).

Preparation of Assay Device

The assay device described above is spotted with dyed reference particles to form an orientation spot. Positive particles, FIV particles, FeLV particles, and negative particles are then also applied. The negative particles are prepared by mixing equal volumes of FeLV negative particles and FIV negative particles.

Assay Protocol

The device is wetted with 0.5 ml of wash solution (2 M potassium chloride, 2.5% non-fat dry milk, 5% BSA, 0.5% Triton® X-100, 0.1 Tween® 80, 0.5% Kathon®, and 0.16 g/l gentamicin) and the prefilter seated in the device. 0.15 ml of conjugate is then placed into a sample tube and 0.2 ml of the sample to be assayed added. The tube is capped and the contents mixed thoroughly by inverting 4-5 times. The tube is then incubated for 3-5 min. at 15°C-30°C and the entire contents of the tube added to the assay device, and allowed to incubate for 3-5 min. at 15°C-30°C. The prefilter is then removed and 0.5 ml of wash solution added and allowed to be absorbed. The assay device is then filled with 2 ml of wash solution. After the wash solution has been absorbed 0.15 ml of TMB substrate (1 g/l tetramethylbenzadine (TMB) in 60% methanol and 40% glycerol) diluted 1:1 in TMB diluent (0.1M dibasic potassium phosphate, 0.1 M citric acid, 30% hydrogen peroxide and 0.1 g/l thimerosal) is added. The device is incubated for 3 min. at 15°C-30°C and the reactionstopped by addition of 0.5 ml of stop solution (0.05-0.1 M ammonium molybdic acid). The result is then read.

ELISA Test for FIV

The following is an example of an ELISA test for FIV antibody in whole blood of a cat. In this test, it is important that the viral antigen be purified sufficiently to be free from host cell proteins in order to reduce background reactions. Generally, purity of the purified virus is confirmed by polyacrylamide gel electrophoresis of the major proteins. The preparation is sufficiently pure when the nucleocapsid or gag gene products, most preferably the p26 nucleocapsid protein, represents at least 5%, preferably 12%-15%, of total protein in the viral preparation. One method for purification of the virus is by density gradient centrifugation in a relatively high ionic strength buffer container glycerol, for example, by collecting the virus at a 50%-90% glycerol interface in 10 mM Tris, 300mM NaCl and 1mM EDTA buffer, pH 7.5, as described above.

In one format of an ELISA test (the test unit is available from Agritech Systems, Portland, ME), FIV antigen is coated, by standard procedures, onto wells in a microtiter dish and incubated with the sample to be tested. Any antibody in the sample specific to FIV forms complexes with the coated viral antigens. Following a wash procedure, an anti-feline horseradish peroxide conjugate is added to the wells such that it binds to feline antibody bound to the FIV antigen coated in the well. In the final step of the assay, unbound anti-feline con-

jugate is washed away and enzyme substrate (hydrogen peroxide) and a chromogen (tetramethylbenzadine, TMB) are added. Subsequent color development is proportional to the amount of specific antibody present in the sample.

The controls include an antigen-coated well treated with 100 µl of a negative control composed of non-FIV reactive feline serum in phosphate buffered saline (PBS, 0.01 M sodium phosphate, 0.15M NaCl, pH 7.2-7.6) containing 5% fetal calf serum (FCS); and at least one antigen coated well treated with 100 µl of a positive control composed of feline anti-FIV antibody positive serum in PBS and FCS.

In the assay, 100 µl of diluted serum or plasma sample is dispensed into each well. The diluent is PBS and FCS, and generally the sample is diluted 100-fold. The wells are incubated for 30 min. at 15°C-30°C, and the liquid contents of all the wells is then aspirated into a waste reservoir and each well is washed five times with approximately 300 µl of diluted wash solution (PBS + 0.05% Tween® 20). The liquid is aspirated from the wells following each wash. Following the final wash, residual wash fluid is removed from the well onto absorbent paper, and 100 µl of anti-feline HRPO conjugate is added to each well (0.1-2.0 µg/ml) and incubated for 30 min. at 15°C-30°C. The wells are then aspirated and washed again. 50 µl of TMB diluent solution is then added to each well, followed by 50 µl of TMB substrate. After incubation for 15 min. at 15°-30°C, 100 µl of stop solution (1 in 400 aqueous dilution of hydrofluoric acid) is added to each well. The absorbance value at 650 nm (A_{650}) is read for the samples and controls. For the assay to be valid, the difference between the positive control and the negative control should be greater than 0.2. In addition, the negative control absorbance should be less than or equal to 0.2. The presence or absence of antibody to FIV is determined by relating the A_{650} value of the sample to the negative control mean. Anything 3 times greater in absorbance intensity than the negative control is regarded as a positive sample.

Deposit

FIV isolate no. 2427 (Petaluma Strain) has been deposited with the ATCC and assigned number CRL 9761, on July 13, 1988.

Claims

1. A method for detecting a feline pathogen antigen and a feline pathogen-specific antibody in a biological sample, comprising:
   (a) providing a feline pathogen antigen which selectively forms a first immune complex with a sample antibody, the antigen being directly bound to a solid support at a first location;
   (b) providing a feline pathogen-specific antibody which selectively forms a second immune complex with a sample antigen, the antibody being directly bound to the solid support at a second location;
   (c) contacting the first location on the support with at least a portion of a biological sample under conditions whereby the first immune complex can form and contacting the second location on the support with at least a portion of such sample of equal concentration under conditions whereby the second immune complex can form;
   (d) washing unbound material from the first and second locations;
   (e) detecting whether the first immune complex is formed by adding a labeled antigen which selectively binds thereto; and
   (f) detecting whether the second immune complex is formed by adding a labeled antibody which selectively binds thereto.

2. A method according to Claim 1 wherein the antigen is FeLV and the antibody is anti-FIV.

3. A method according to Claim 1 wherein the antigen is FIV and antibody is anti-FeLV.

4. A solid support for use in a method according to any preceding Claim including a feline pathogen antigen and a feline pathogen-specific antibody, wherein the antigen is directly bound to the solid support at a first location and the antibody is directly bound to the solid support at a second location.

5. A solid support according to Claim 4 and comprising one of a microtitter well, a glass or plastic bead, a filter matrix, a polystyrene latex bead, or other microparticles.

6. A solid support according to Claim 4 or Claim 5 wherein the antigen is FIV, a polypeptide comprising an epitope of FIV, or derived from FIV, and the antibody is anti-FeLV.

7. A solid support according to Claim 4 or Claim 5 wherein the antigen is FeLV, a polypeptide comprising an epitope of FeLV, or derived from FeLV, and the antibody is anti-FIV.

8. A kit comprising:
   (a) a solid support comprising a feline pathogen antigen capable of selectively forming a first immune complex with a sample

antibody, which antigen is directly bound to a solid support at a first location; and a feline pathogen-specific antibody capable of selectively forming a second immune complex with a sample antigen, which antibody is directly bound to the solid support at a second location, wherein the antigen and the antibody are each capable of forming separately detectable immune complexes with biological samples of equal concentration;
(b) means for detecting said first complex comprising a labeled antigen which selectively binds thereto; and
(c) means for detecting said second immune complex comprising a labeled antibody which selectively binds thereto.

9. A kit according to Claim 8 wherein the antigen is FIV, a polypeptide comprising an epitope of FIV, or derived from FIV, and the antibody is anti-FeLV.

10. A kit according to Claim 8 wherein the antigen is FeLV, a polypeptide comprising an epitope of FeLV, or derived from FeLV, and the antibody is anti-FIV.

11. A kit according to any of Claims 8 to 10 wherein the solid support has a region including one or both of a positive and a negative control spot.

Patentansprüche

1. Verfahren zum Nachweis eines Katzenkrankheitserreger-Antigens und eines für einen Katzenkrankheitserreger spezifischen Antikörpers in einer biologischen Probe, darin bestehend, daß
(a) ein Katzenkrankheitserreger-Antigen erhalten wird, das selektiv einen ersten Immunkomplex mit einem Probenantikörper bildet, wobei das Antigen direkt an einen festen Träger an einer ersten Stelle gebunden wird;
(b) ein für einen Katzenkrankheitserreger spezifischer Antikörper erhalten wird, der selektiv einen zweiten Immunkomplex mit einem Probenantigen bildet, wobei der Antikörper direkt an den festen Körper an einer zweiten Stelle gebunden wird;
(c) die erste Stelle auf den Träger mit wenigstens einem Teil einer biologischen Probe unter Bedingungen, unter denen sich der erste Immunkomplex bilden kann, und die zweite Stelle auf dem Träger wenigstens mit einem Teil der Probe gleicher Konzentration unter Bedingungen, unter denen sich der zweite Immunkomplex bilden kann, in Berührung gebracht werden;
(d) ungebundenes Material von der ersten und der zweiten Stelle gewaschen wird;
(e) durch Zugabe eines markierten Antigens festgestellt wird, ob der erste Immunkomplex gebildet wird, an den es sich selektiv bindet; und
(f) durch Zugabe eines markierten Antikörpers festgestellt wird, ob der zweite Immunkomplex gebildet wird, an den er sich selektiv bindet.

2. Verfahren gemäß Anspruch 1, dadurch gekennzeichnet, daß das Antigen FeLV und der Antikörper Anti-FIV ist.

3. Verfahren gemäß Anspruch 1, dadurch gekennzeichnet, daß das Antigen FIV und der Antikörper Anti-FeLV ist.

4. Fester Träger für die Verwendung bei einem Verfahren gemäß den vorhergehenden Ansprüchen mit einem Katzenkrankheitserreger-Antigen und einem für einen Katzenkrankheitserreger spezifischen Antikörper, worin der Antikörper direkt an den festen Träger an einer ersten Stelle und der Antikörper direkt an den festen Träger an einer zweiten Stelle gebunden wird.

5. Fester Träger gemäß Anspruch 4 und mit einer Mikrotiter-Vertiefung, einer Glas-oder Kunststoffperle, einer Filtermatrix, einer Polystyrollatexperle oder anderen Mikropartikeln.

6. Fester Träger gemäß Anspruch 4 oder Anspruch 5, worin das Antigen FIV, ein Polypeptid mit einem Epitop des FIV oder von FIV abgeleitet, und der Antikörper Anti-FeLV ist.

7. Fester Träger gemäß Anspruch 4 oder Anspruch 5, worin das Antigen FeLV, ein Polypeptid mit einem Epitop des FeLV oder vom FeLV abgeleitet, und der Antikörper Anti-FIV ist.

8. Testbesteck, bestehend aus:
(a) einem festen Träger mit einem Katzenkrankheitserreger-Antigen, das selektiv einen ersten Immunkomplex mit einem Probenantikörper bilden kann, wobei das Antigen direkt an einen festen Träger an einer ersten Stelle gebunden wird; und mit einem für einen Katzenkrankheitserreger spezifischen Antikörper, der selektiv einen zweiten Immunkomplex mit einem Probenantigen bilden kann, wobei der Antikörper direkt an

den festen Träger an einer zweiten Stelle gebunden wird, worin das Antigen und der Antikörper jeweils getrennt nachweisbare Immunkomplexe mit biologischen Proben gleicher Konzentration bilden können;
(b) Mittel zum Nachweis des ersten Immunkomplexes mit einem markierten Antigen, das sich selektiv daran bindet; und
(c) Mittel zum Nachweis des zweiten Immunkomplexes mit einem markierten Antikörper, der sich selektiv daran bindet.

9. Testbesteck gemäß Anspruch 8, worin das Antigen FIV, ein Polypeptid mit einem Epitop des FIV oder vom FIV abgeleitet, und der Antikörper Anti-FeLV ist.

10. Testbesteck gemäß Anspruch 8, worin das Antigen FeLV, ein Polypeptid mit einem Epitop des FeLV oder vom FeLV abgeleitet, und der Antikörper Anti-FIV ist.

11. Testbesteck gemäß den Ansprüchen 8 bis 10, worin der feste Träger eine Region besitzt, die einen positiven und einen negativen Kontrolltüpfel oder beide enthält.

Revendications

1. Procédé de détection d'un antigène pathogène des félins et d'un anticorps spécifique de pathogène des félins dans un échantillon biologique comprenant les étapes consistant à :
(a) fournir un antigène pathogène des félins qui forme de façon sélective un premier complexe immun avec un anticorps de l'échantillon, l'antigène étant directement lié à un support solide au niveau d'un premier emplacement ;
(b) fournir un anticorps spécifique de pathogène des félins qui forme de façon sélective un deuxième complexe immun avec un antigène de l'échantillon, l'antigène étant directement lié au support solide au niveau d'un deuxième emplacement ;
(c) mettre en contact le premier emplacement du support avec au moins une partie d'un échantillon biologique dans des conditions telles que le premier complexe immun peut se former et mettre en contact le deuxième emplacement du support avec au moins une partie d'un tel échantillon de concentration égale dans des conditions telles que le deuxième complexe immun peut se former ;
(d) laver le produit non lié à partir des premier et deuxième emplacements ;
(e) détecter si le premier complexe immun est formé par addition d'un antigène marqué qui se lie de façon sélective ; et
(f) détecter si le deuxième complexe immun est formé par addition d'un anticorps marqué qui se lie de façon sélective.

2. Procédé selon la revendication 1, dans lequel l'antigène est FeLV et l'anticorps est anti-FIV.

3. Procédé selon la revendication 1, dans lequel l'antigène est FIV et l'anticorps est anti-FeLV.

4. Support solide à utiliser dans un procédé selon l'une quelconque des revendications précédentes, comprenant un antigène pathogène des félins et un anticorps spécifique de pathogène des félins, dans lequel l'antigène est directement lié au support solide au niveau d'un premier emplacement et l'anticorps est directement lié au support solide au niveau d'un deuxième emplacement.

5. Support solide selon la revendication 4, comprenant un élément parmi un puits de microtirage, une bille de verte ou de plastique, une matrice filtrante, une bille de latex polystyrénique et d'autres microparticules.

6. Support solide selon la revendication 4 ou la revendication 5, dans lequel l'antigène est FIV, un polypeptide comprenant un déterminant antigénique de FIV ou est dérivé de FIV et l'anticorps est anti-FeLV.

7. Support solide selon la revendication 4 ou la revendication 5, dans lequel l'antigène est FeLV, un polypeptide comprenant un déterminant antigénique de FeLV ou est dérivé de FeLV et l'anticorps est anti-FIV.

8. Kit comprenant :
(a) un support solide comprenant un antigène pathogène des félins capable de former de façon sélective un premier complexe immun avec un anticorps de l'échantillon, lequel antigène est directement lié à un support solide au niveau d'un premier emplacement ; et un anticorps spécifique de pathogène des félins capable de former de façon sélective un deuxième complexe immun avec un antigène de l'échantillon, lequel anticorps est directement lié au support solide au niveau d'un deuxième emplacement, dans lequel l'antigène et l'anticorps sont chacun capables de former des complexes immuns détectables de façon séparée avec des échantillons biologiques de

concentration égale ;
(b) un moyen de détection dudit premier complexe comprenant un antigène marqué qui se lie de façon sélective ; et
(c) un moyen de détection dudit deuxième complexe immun comprenant un anticorps marqué qui se lie de façon sélective.

9. Kit selon la revendication 8, dans lequel l'antigène est FIV, un polypeptide comprenant un déterminant antigénique de FIV ou est dérivé de FIV et l'anticorps est anti-FeLV.

10. Kit selon la revendication 8, dans lequel l'antigène est FeLV, un polypeptide comprenant un déterminant antigénique de FeLV ou est dérivé de FeLV et l'anticorps est anti-FIV.

11. Kit selon l'une quelconque des revendications 8 à 10, dans lequel le support solide possède une région comprenant un ou deux éléments parmi un point témoin positif et un point témoin négatif.

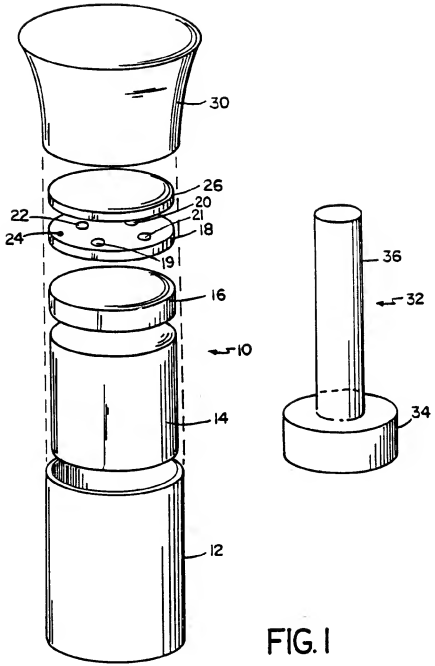

FIG. 1